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SENSORY DEPRIVATION IN THE CRICKET NERVOUS SYSTEM: EVIDENCE FOR A CRITICAL PERIOD

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INTRODUCTION

Studies of the vertebrate visual system have supported the hypothesis that the response properties of mature neurones are partially determined by their functional history. Hubel & Wiesel (1963) found that the functional organization of the cat visual cortex was sensitive to the pattern of sensory input from the two eyes. They also demonstrated that this sensitivity of the visual cortex to experimental manipulations was restricted to a well defined period early in post-embryonic development which they called the 'critical period' (Hubel & Wiesel, 1970). During this critical period the visual cortex could be extensively modified by the experience it received. Experience outside of the critical period had little or no effect on the organization of the system (Blakemore & Van Sluyters, 1974; Hubel & Wiesel, 1970).

We recently reported that development of the response characteristics of an identified interneurone in an invertebrate nervous system was affected by the sensory stimulation the animal receives during development (Matsumoto & Murphey, 1977*a*). When sensory activity was lowered throughout post-embryonic development the responsiveness of the identified neurone in adults was below normal. The present study sought to determine whether this sensitivity to sensory input was restricted to one or another developmental stage.

The cercal-to-giant interneurone system of crickets consists of a bilaterally symmetric pair of abdominal appendages, namely the cerci, and a group of a dozen or so bilaterally homologous first order interneurones. The cerci are highly specialized as mechanosensitive organs. Afferent input from sensory cells associated with mechanosory hairs located on the cerci innervate two interneurones known as the lateral and medial giant interneurones (Palka & Edwards, 1974; Murphey, 1973; Murphey, Matsumoto & Mendenhall, 1976). Blocking movement of the cercal hairs drastically lowers afferent input to these two interneurones.

Crickets are hemimetabolous insects, and therefore mature through a series of stages (instars) before moulting to the sexually mature adult instar. A full complement of central neurones is present at the time of hatching but peripheral sensory neurones are added at each moult (Gymer & Edwards, 1967). Since neurones are being added during larval life it was of interest to examine the effects of restricted sensory input during these developmental stages. Since the cerci are accessible to experimental

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manipulation immediately upon hatching and during each instar, a blockade of sensory input to the interneurones can be imposed by blocking hair movement on one or both cerci during any chosen instar, or instars. When the specimen moults to the next instar this block is relieved.

We have found that the response properties of the medial giant interneurones are most affected by deprivation during the first half of larval development and are progressively less influenced by treatments during the later stages of development.

METHODS

Sensory deprivation. Sensory input from the cerci to the giant interneurones was eliminated by immobilizing the mechanoreceptive hairs with a film of facial cleansing cream (Clinique Cleansing Cream). In the previous experiments the specimens were examined 24 hr after the application of the cream to determine the efficacy of the treatment (Matsumoto & Murphey, 1977*a*). A complete block of all receptor hairs is crucial since a very small number of functional receptors is sufficient to activate the giant interneurones and eliminate any deprivation effects.

At each ecdysis the receptor hairs are free to move and must be immobilized again if further deprivation is necessary. The specimens were monitored daily for signs of the onset of ecdysis. Twenty-four hours after the first moulted specimen in a sibling group was detected the entire group was anaesthetized on ice and those specimens that had moulted were treated. The remaining specimens were kept for an additional 24 hr. Those not moulting after this period were discarded. The length of the instars for specimens reared at 25 °C with 12 : 12 light–dark cycle is shown in Table 1. Note that the duration of the later instars is longer than the earlier ones. Repeated cooling to treat or carefully examine specimens extends each instar by 1–2 days when compared with untreated controls. The various deprivation paradigms and their designated titles are shown in Text-fig. 1.

TABLE 1. Duration of instars

	Instar									
	1	2	3	4	5	6	7	8	9	Adult
Duration (days)	6·0	6·0	6·0	7·0	6·8	7·8	8·0	8·9	9·8	4 months+
Age in days at the time of ecdysis	6	12	18	25	32	40	48	57	67	

Duration of instars for specimens reared at 25 °C on a 12 : 12 light–dark cycle. Values represent averages for a number of groups.

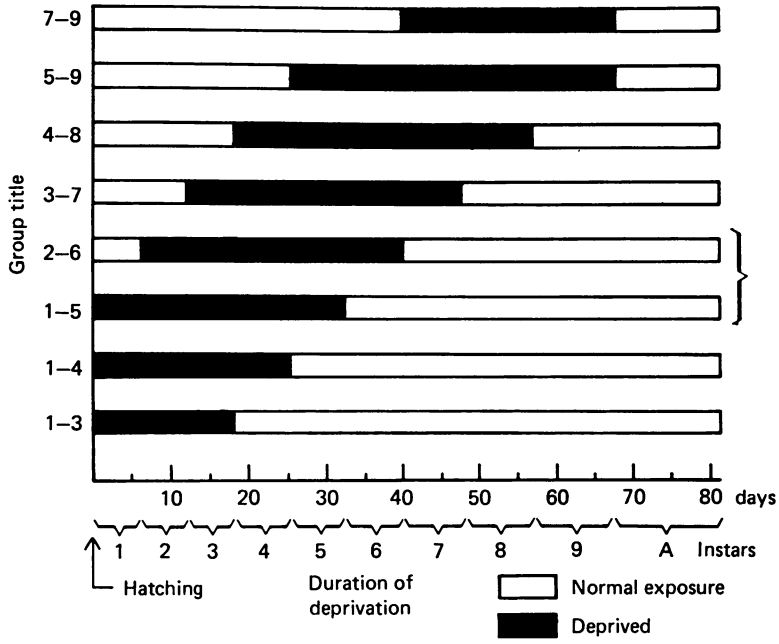
Intracellular recording. The method of intracellular recording has been described elsewhere (Murphey, 1973; Murphey & Matsumoto, 1976; Matsumoto & Murphey, 1977*b*). Briefly, the specimens were anaesthetized by cooling them on ice for 10–15 min. The C.N.S. was then exposed from the dorsal side and a holder manipulated under the terminal abdominal ganglion for stability. Ten minutes after the dissection was complete, the medial giant interneurones were impaled. The order of impalement was alternated between preparations to control for variability in the responsiveness of the neurones due to the effects of anaesthetization and dissection. Intracellular recording periods of long duration (1 hr or longer) or reimpalement of the neurones often resulted in a progressive increase in the sensitivity of the neurone. Therefore all data reported here was collected within a few minutes of impalement and data from reimpaled neurones were discarded.

Quantitative methods. Intensity–response curves were determined for both medial giant interneurones in each specimen. The number of action potentials elicited from each neurone was determined in ten stimulus trials at each of four stimulus intensities (75, 80, 85, 90 db). The relationship is approximately linear when the response is plotted against the intensity in decibels (e.g. Text-fig. 4). A least-squares line of best fit was then calculated for each medial in

each specimen. The slopes of treated and control neurones were then compared using a *t* test. Differences between groups were assessed by comparing the ratios of the slopes (control/treated) in the two groups being considered. Again a *t* test was used to compare these ratios.

Extracellular recording. The extracellular recording methods have been described in detail elsewhere (Edwards & Palka, 1974; Murphey, Mendenhall, Palka & Edwards, 1975). The advantage of this recording method is the long-term stability of the recordings and mobility of the preparation. The disadvantage is that the two largest neurones cannot always be distinguished from one another, although they can be distinguished from all other neurones (Murphey & Matsumoto, 1976; Murphey, Palka & Hustert, 1977).

Stimulation. The sound stimulus consisted of pure tones generated by a Heath model EU-BIA function generator regulated by an electronic switch (Grason-Stadler Model 1287B). The switch turned the signal on and off with a constant rise time without producing transients or other stimulus artifacts.

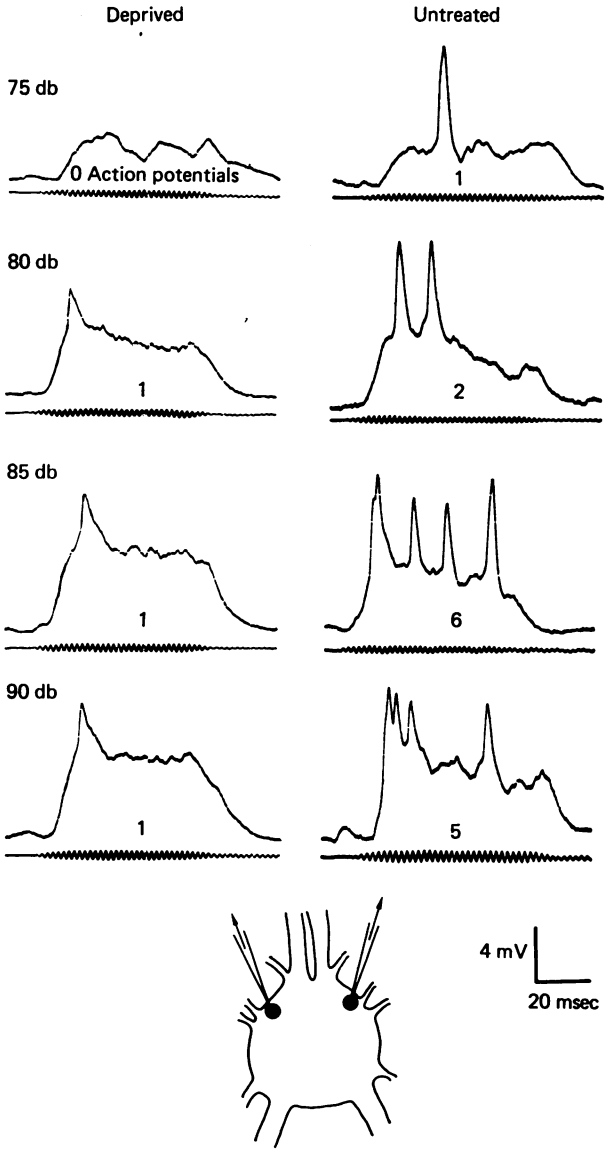


Text-fig. 1. Schematic representation of the treatments used in this study. Sensory deprivation is indicated by the filled region of the bar, normal exposure by an open bar. The maximum effect was obtained in groups labelled 1–5 and 2–6 (bracket at right of Figure). Group titles, used throughout the text, refer to the instars treated. Note that all specimens were tested 1–2 weeks after the imaginal moult.

RESULTS

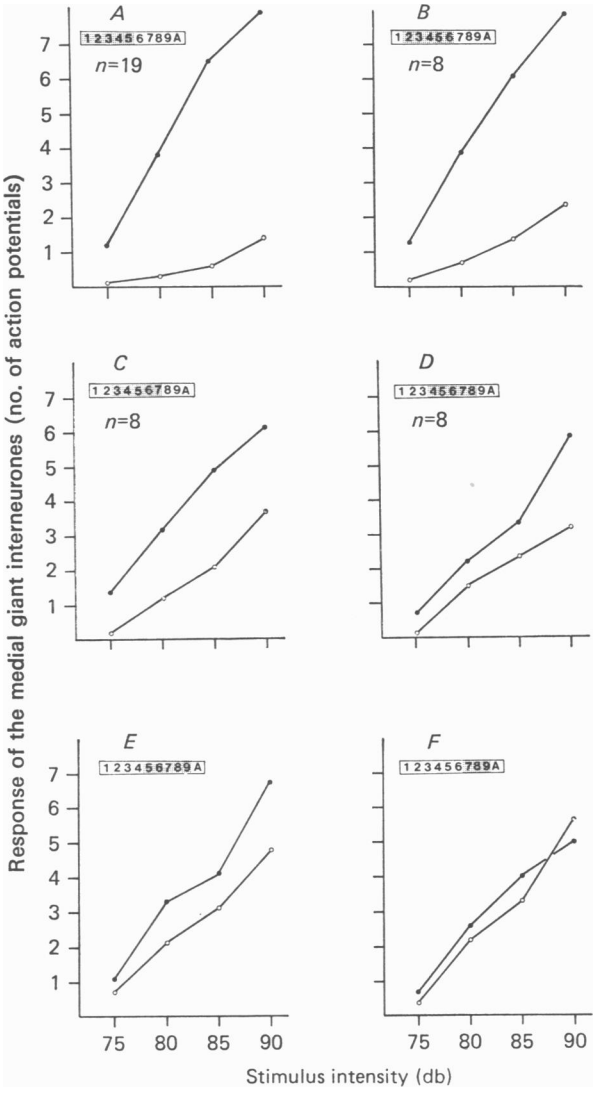
The effect of time of initiation of deprivation on the response properties of the medial giant interneurone. It was reported in an earlier study that chronic sensory deprivation of the cercal sensory system resulted in a decrease in the responsiveness of the medial giant interneurone (Matsumoto & Murphey, 1977*a*). In that study the specimens were deprived throughout the first seven to eight larval instars. In order to determine whether the central nervous system exhibited periods of differential sensitivity to the effects of deprivation, specimens were treated for a constant number of instars (five) but the treatment was initiated at varying times during development.

The results of varying the time of onset of the deprivation indicate that the dependence of the nervous system on sensory input is not constant throughout post-larval life. When sensory deprivation commenced at hatching and lasted five larval instars (35 days) the responsiveness of the treated neurone was lowered. (Throughout the text the neurone whose primary source of excitation is blocked is called the 'treated' neurone, its contralateral homologue is called the 'control' or 'untreated'



Text-fig. 2. Sample recordings from the soma of the right (deprived) and left (untreated) medial giant interneurone in one experimental specimen. The right neurone was deprived for the first five larval instars. The deprived neurone was less sensitive to standard stimuli compared to the untreated one at all stimulus intensities. The inset shows the recording situation.

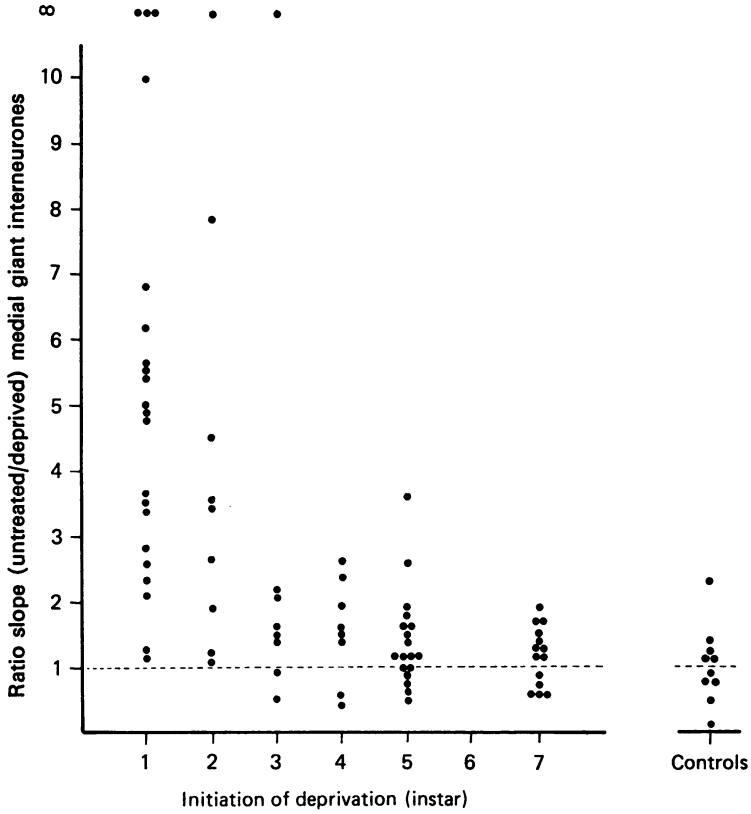
neurone.) Intracellular recordings were obtained from both medial giant interneurones in each individual, and sample recordings are illustrated in Text-fig. 2. Note that the treated neurone is much less sensitive to tones than the control neurone. The responses of the deprived and untreated medial giant interneurones of nineteen specimens tested in this manner are summarized in Text-fig. 3*A*. The response of the deprived neurones was significantly lower than the untreated ones (*t* test; $P < 0{\cdot}001$). This effect is similar to that reported previously when the treatment



Text-fig. 3. Intensity response curves were obtained from medial giant interneurones when unilateral treatment was initiated at different developmental stages and had a constant duration. *A*, deprived first to fifth instars. *B*, deprived second to sixth instars. *C*, deprived third to seventh instars. *D*, deprived fourth to eighth instars. *E*, deprived fifth to ninth instars. *F*, deprived seventh to ninth instars. The inset in each panel indicates the duration of deprivation (shaded area) in instars. All data were obtained intracellularly. Untreated neurone (filled circles); deprived neurone (open circles).

lasted eight instars (Matsumoto & Murphey, 1977*a*). In a similar fashion, deprivation initiated in the second instar (Text-fig. 1, group 2–6) altered the response of the treated medial giant interneurone in a manner identical to that obtained when treatment was initiated at hatching (Text-fig. 3*B*).

In contrast, when the deprivation paradigm was imposed beginning in the third instar the extent of depression in the responsiveness of medial giant interneurone

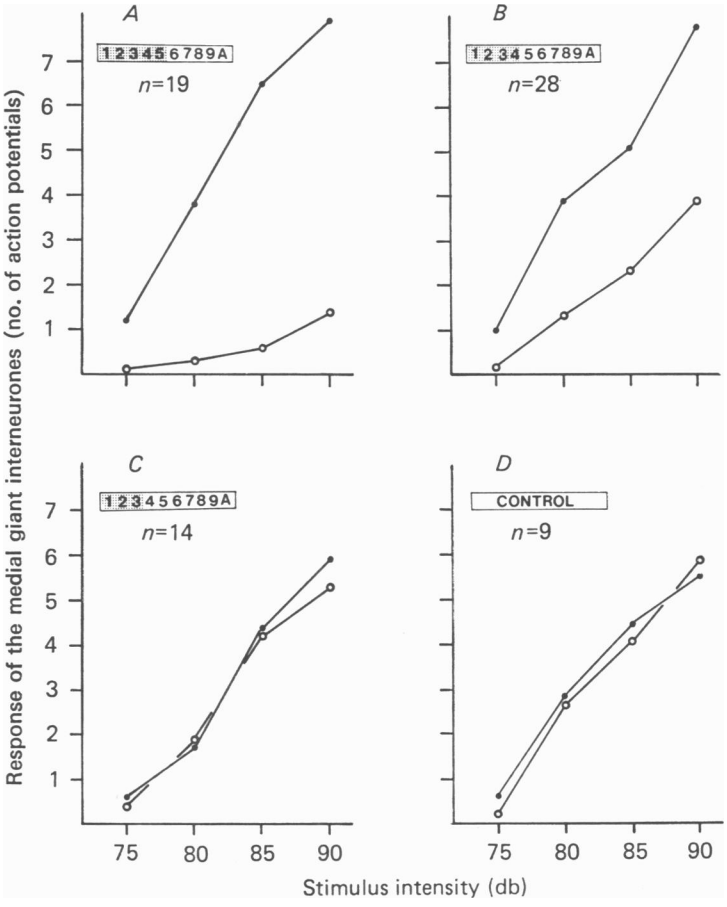


Text-fig. 4. The relationship between the time of initiation of the deprivation and the strength of the effect. The difference between the two neurones in each specimen is expressed as the ratio of the slope of the intracellular response of the control neurone, tested at increasing stimulus intensities, to the slope of the response of the deprived neurone over the same intensity range. A ratio of 1·0 (interrupted line) indicates no difference. Values greater than 1·0 indicate a deprivation effect. The data points above the maximal scale indicate that the deprived medial giant interneurone did not respond at all to the sound stimulus. Each data point represents one specimen.

appeared to be less than the previously described groups although it was still significant (Text-fig. 3*C*) (*t* test; $P < 0{\cdot}05$). Five of eight specimens in this group did not show any difference between the deprived and untreated interneurones. Deprivation initiated in the fourth instar or later had no significant affect on the response (Text-fig. 3*D*, *E* and *F*).

The relationship between the onset of the deprivation and the modification in response sensitivity of the deprived interneurone is represented graphically in Text-

fig. 4. A stimulus–response curve was obtained for both deprived and untreated types of each specimen. The difference between the deprived and untreated medial giant interneurones was expressed as a ratio of the slopes of the intensity–response curves (untreated/deprived). A ratio near 1·0 indicated no difference between the two neurones while larger numbers indicate a reduction in the response sensitivity of the deprived one. The percentage of specimens showing a depression in responsiveness and the degree of depression observed decreased the later the deprivation

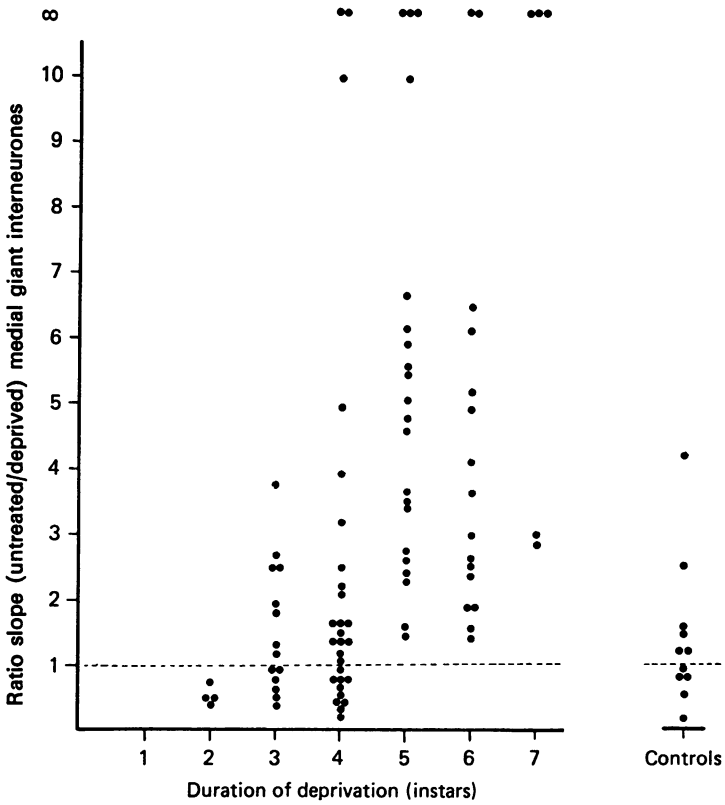


Text-fig. 5. The effect of treatment duration on the response properties of the medial giant interneurone. Intensity response curves for treated and control neurones for different durations of treatment. *A*, the response of the neurones in specimens treated during the first five instars (same as Text-fig. 4*A*). *B*, the response of the neurones in specimens treated during the first four instars. *C*, the response of the neurones in specimens treated during the first three instars. *D*, the response of the neurones in control specimens. See text for details. Untreated, filled circles; deprived, open circles.

treatment was initiated. In summary, the results of Text-figs. 3 and 4 indicate that the development of the cercal sensory system is modified by altered levels of sensory input during the early instars and is relatively insensitive in later instars.

The effect of duration of deprivation on the response properties of the medial giant

interneurone. The effects of shorter periods of sensory deprivation during the sensitive period were also studied. When deprivation was initiated at hatching and continued through the fourth instar (24 days) the responsiveness of the treated neurone was significantly different from the untreated one (Text-fig. 5*B*, $P < 0{\cdot}05$). Specimens deprived for shorter periods of time, two or three instars (12–18 days) were not significantly different from controls (Text-figs. 5*C* and *D* and 6). It is apparent that

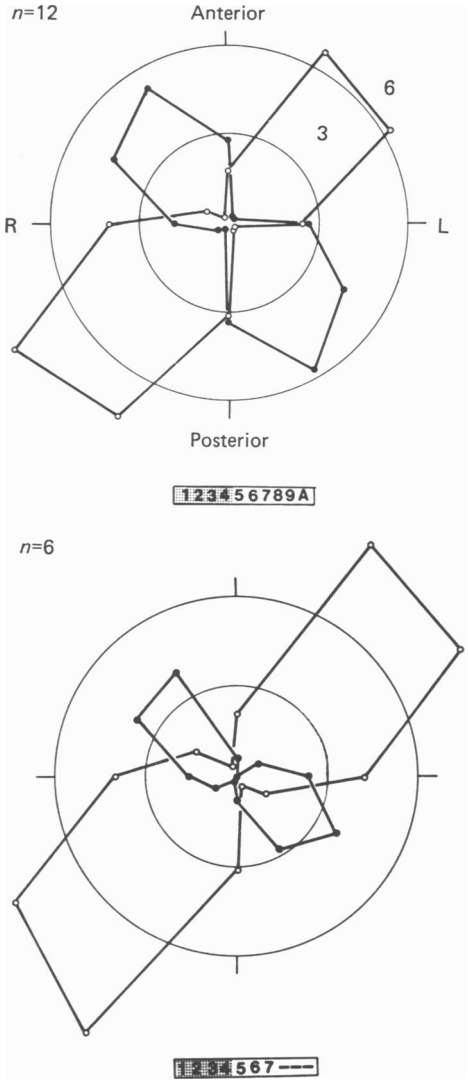


Text-fig. 6. The relationship between the duration of the deprivation and the response sensitivity of the medial giant interneurone. The difference between the two neurones in each specimen is expressed as the ratio of the slope of the intracellular response of the control neurone, tested at increasing stimulus intensities, to the slope of the response of the deprived neurone over the same intensity range. A ratio of 1·0 (interrupted line) indicates no difference. Values greater than 1·0 indicate a deprivation effect. The data points above the maximal scale indicate that the deprived neurone did not respond at all to the sound stimulus (i.e. maximal depression). Each data point represents one specimen.

longer periods of deprivation affected neuronal response properties more than do shorter periods. Both the number of specimens exhibiting the affect and the strength of the affect are increased by increased durations of treatment.

The effect of treatment on afferent survival. In our previous study we ruled out the possibility that the procedures used to deprive the specimen of sensory input resulted in degeneration of sensory neurones. That study examined adult cercal nerves after varying periods of treatment (Matsumoto & Murphey, 1977*a*). In order

to control for the possibility that the cerci of very young animals were more sensitive to damage by application of the cleansing cream, we examined the cercal nerve of first and second instar specimens after treatment. If the treatment killed



Text-fig. 7. Recovery of response properties due to normal exposure following the treatment. Specimens were deprived for the first four instars and were then tested 20 days later (in the seventh instar) or 50 days later (as adults). The results were obtained using extracellular recording methods. Note that the asymmetry between treated and control neurones is greater in the seventh instar than in the adult suggesting that some recovery is occurring.

sensory neurones, we ought to have detected degeneration only in the treated cercal nerve. We examined the cercal nerves of four first and second instar specimens 8–24 hr after treatment. Three exhibited no degeneration in either cercal nerve (Pl. 1)

(six to ten sections/nerve, per specimen). Degeneration was detected in the cercal nerve on the treated side as well as on the untreated side of the remaining specimen and therefore could not be attributed to the treatment. We conclude that sensory cells do not die as a result of treatment.

Recovery from early deprivation. The experiments described thus far include an uncontrolled variation in the duration of normal exposure following the last treatment. The possibility that the giant interneurones could recover from the effects of deprivation following treatment was therefore examined. Animals deprived for the first four instars were either tested as adults after a long recovery period or as juveniles (seventh instar) after shorter recovery periods (Text-fig. 7). The response of the treated and control neurones for each specimen was expressed as a ratio and those tested in the seventh instar were compared with those tested in the adult instar. The mean ratio (untreated/treated) was significantly larger in the specimens tested in the seventh instar than in the specimens tested in the adult. Apparently the effects of deprivation are reversed to some extent by normal activity. A more detailed study of the effects of recovery is in progress.

DISCUSSION

Evidence for a critical period. Our results demonstrate that there is a period early in post embryonic development when the C.N.S. of crickets is affected by the sensory input which the animal receives. When the duration of deprivation was held constant but the time of initiation was altered it was found that deprivation initiated early (first or second instar) had a more powerful effect on development than deprivation initiated late (third instar or later). The effects were graded; the later that deprivation was initiated the less of an effect it had on interneurone response properties (Text-fig. 4). The duration of deprivation was also an important variable. To produce detectable effects deprivation had to last a minimum of 24 days (four instars); shorter treatments had no effect. Taken together these results suggest that there is a period of maximum susceptibility which extends from day 6 to day 47 (second to sixth instar). It is difficult to dissect this period further because at least 24 days of deprivation are required to obtain a detectable difference in responsiveness of the treated and non-treated medial giant interneurone.

The importance of the first few instars in normal development is consistent with our previous work. We have demonstrated that certain aspects of the circuitry of the cercal-to-giant interneurone system are more susceptible to periods of deafferentation early in development than later in development (Murphey *et al.* 1975). This system is still developing after hatching and our treatments seem to disrupt normal wiring of the system. Late treatments on the other hand are imposed on a C.N.S. which is virtually complete and therefore they have no effect.

At least two variables require further attention since they remain essentially uncontrolled in these experiments; the duration of normal exposure before deprivation and the length of time between the end of treatment and testing of the specimens. Both of these variables are changing even when the duration of treatment is constant (Text-fig. 1), and the length of normal exposure after treatment is different for all but one pair of treatments. The effects of normal exposure prior to treatment are

difficult to assess since in most cases normal exposure prior to deprivation exactly parallels the time of initiation of treatment.

The possibility that recovery of some sort occurs due to normal exposure following treatment was briefly examined experimentally. Specimens treated for four instars were tested in the seventh instar, as well as in the adult instar. The effects of deprivation were stronger in the seventh instar than in the adult. This suggests that normal exposure following some treatment paradigms may mediate recovery. This fact does not alter our conclusions since groups which show the deprivation effect (e.g. group 1–5, Text-fig. 1) have a relatively long period of normal exposure following treatment while other groups which exhibit no deprivation effect received only brief periods of normal exposure following treatment (e.g. group 5–9, Text-fig. 1). Thus while recovery of function may be a factor in these experiments the errors introduced would reduce the observed differences rather than enhance them.

Possible effects of injury. We considered the possibility that the difference between early and late treatments is a mechanical effect. The cercus might be more susceptible to injury in the early instars than in later instars. The depression in response sensitivity of the medial giant interneurone would then be the result of the degree of damage to the cercus. However, the deprivation procedure does not cause the loss of sensory receptors nor a modification in their response characteristics in later instars (Matsumoto & Murphey, 1977*a*). Nor does it cause degeneration in early instars (Pl. 1). Further, complete cercal deafferentation for a single instar during this early period (i.e. first or second instar) does not result in altered neuronal responses (S. G. Matsumoto, in manuscript). Therefore, the damage to the cercus by the deprivation treatment would have to be extremely severe to explain the observed effects.

Conclusion. This study examined the post-embryonic development of the central nervous system of the cricket for its sensitivity to an imbalance in sensory stimulation. We found that the susceptibility of the cercal sensory system to deprivation decreased with age. Progressively later treatments resulted in less change in the response properties of medial giant interneurones.

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EXPLANATION OF PLATE

The afferent axons do not degenerate as a result of treatment. Cross-sections of the right (lower) and left (upper) cercal nerves of a specimen whose right cercus was treated with cleansing cream 24 hr before fixation. No degenerating axons appear in these or adjacent sections.